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Improving Aerosol Drug Delivery in CF therapy

S. Seemann, A. Schmitt, R. Waldner, M. Hug, M. Knoch
PARI Aerosol Research Institute, Munich, Germany

Aims: The PARI LC PLUS® jet nebulizer is the approved delivery system for TOBI® (300 mg tobramycin in 5mL) to treat pseudomonas aeruginosa infections in CF patients. The eFlow®rapid electronic nebulizer (PARI GmbH) has been adopted from the eFlow® delivery platform and optimized for administration of currently approved medications used in CF. This study was undertaken to investigate the *in-vitro* aerosol characteristics of the eFlow®rapid with TOBI® compared to the LC PLUS®.
Methods: Delivered (DD) and Respirable Doses (RD) were measured during simulated breathing (tidal volume 500 ml, 15 breaths/min, inhal./exhal. ratio 1:1). The droplet size distribution was assessed by laser diffraction (Malvern MasterSizerX) at a constant flow of 20 l/min.
Results: DD and RD are both higher with eFlow®rapid compared to LC PLUS®, however, drug mass of coarse (> 5µm) and very fine droplets (< 2µm) is 17% and 57% less for eFlow®rapid vs. LC PLUS®. This indicates higher lung deposition at the desired site of action and reduced side effects due to lower oropharyngeal deposition and systemic absorption. Respirable Drug Delivery Rate is 2.6-fold higher, thus allowing for a significantly shorter nebulization time with TOBI®/eFlow®rapid (6.8min vs. 13.3min).

	eFlow®rapid Mean	PARI LC PLUS® Mean
DD [mg]	131.1	114.2
RD [mg < 5 µm]	95.2	71.7
[mg < 2 µm]	8.7	20.1
[mg > 5 µm]	35.8	43.1
RDDR [mg < 5 µm/min]	14.0	5.4
Nebulization Time [min]	6.8	13.3
MMD [µm]	3.89	4.00
GSD	1.53	2.00
RF [% < 5µm]	73.1	62.3

Conclusions: The eFlow®rapid electronic nebulizer shows a high RDDR and significantly reduced treatment time with TOBI® (5 ml volume fill) which is essential for an effective and convenient inhalation therapy. This is important with respect to patient compliance and, thereby, may improve therapeutic efficacy. Other medications used in CF will be further investigated.

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Azithromycin (AZM) inhibits Nuclear Factor-kB (NF-kB) activity and expression of interleukin 8 (IL8) in CF airway epithelial cells

C. Cigana, E. Nicolis, M. Pasetto, B.M. Assael, P. Melotti
Cystic Fibrosis Center-Azienda Ospedaliera di Verona, Verona-Italy

CF airways are characterized by exaggerated inflammation. This condition correlates with activation of the NF-kB pathway associated with production of IL8, the major polymorphonuclear leukocyte chemokine in the lung. Higher IL8 levels are described in CF than in non-CF cells. AZM has been reported to ameliorate airway inflammation in CF patients. We previously demonstrated that AZM inhibits IL8 expression in a CF cell line.
This study was aimed to investigate whether AZM affects NF-kB activity in the same cells and whether the same effects are detectable in another cell line.
IL-8 mRNA levels were determined by quantitative real-time PCR based analysis. Amounts of IL-8 secreted were detected in supernatants by ELISA.
We have found that AZM is capable of reducing constitutive IL-8 mRNA expression as well as protein secretion in both CF cell lines.
In this study we found an about 40% reduction of IL-8 mRNA expression levels (n=9, p<0.05) and an about 50% reduction of IL8 release (n=4, p<0.05).
Since IL-8 is considered a NF-kB target gene, we then hypothesized an eventual effect of AZM on the activation of this transcription factor in both cell lines. NF-kB activity was evaluated as DNA binding by an ELISA-based format assay. We determined a statistically significant reduction of about 50% of DNA binding activity in the presence of AZM in both cell lines.
These results support the anti-inflammatory role of this macrolide. In particular inhibition of NF-kB activity suggests other pro-inflammatory genes regulated by this transcription factor as potential target of AZM relevant for therapy of CF.

Supported by Italian Cystic Fibrosis Research Foundation and Legge 548/93 Finanziamento Ricerca Fibrosi Cistica 2004.

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Is the GCC repeat polymorphic length in the Multidrug resistance-associated protein 1 (MRP1) gene relevant for regulating transcriptional activity constitutively and in response to azithromycin (AZM)?

E. Nicolis, M. Pasetto, C. Cigana, U. Pradal, B.M. Assael, P. Melotti
Cystic Fibrosis Center-Azienda Ospedaliera, Verona-Italy

MRPs exhibit homology with CFTR, which is defective in CF. Higher levels of MRP1 mRNA have been associated to less severe CF phenotype as well as to beneficial effects of AZM in CF patients. *MRP1* has been considered either as potential modifier gene and/or as novel target for pharmacotherapy of CF, in particular for AZM. We previously described a polymorphic length of the GCC repeat in the 5'untraslated region of the *MRP1* gene (alleles ranging from 7 to 14 triplets).
Since the repeat is in a region involved in regulation of *MRP1* expression we hypothesized a functional relevance of this polymorphism.
In this study we compared the transcriptional activity of the proximal *MRP1* 5'regulatory region containing 7 or 14 GCC triplets. To this end gene reporter studies were performed in a CF airway epithelial cell line in basal conditions and in the presence of AZM. The two alleles do not show statistically significant differences in transcriptional activity; AZM treatment does not significantly affect transcriptional activity.
In our experimental conditions the sequence responsible for variable levels of *MRP1* mRNA detected in other studies seems not to be included in the DNA sequence we analyzed. However, we still can not exclude a functional relevance of this polymorphism on *MRP1* expression. Additional sequences as well as conditions to whom the cells can be exposed *in vivo* that can have an effect on *MRP1* expression remain to be investigated. Our experimental model might be useful for testing other molecules potentially relevant for therapy of CF.

Supported by Italian Cystic Fibrosis Research Foundation and Legge 548/93 Finanziamento Ricerca Fibrosi Cistica 2004.

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Long-term low-dose therapy with macrolides in bronchopulmonary disease in Cystic Fibrosis (CF) pediatric patients

A.M. Radionovitch[1], N.J. Kashirskaya[2], N.I. Kapranov[3]
Research Centre for Medical Genetics RAMS, Moscow, Russia

The aim was to evaluate the potential effect of long-term administration of macrolides on clinical, functional and laboratory parameters, such as FVC, FEV1, weight/height index (Wt/Ht%), Schwachman and Brasfield scores (SBs), chest X-ray, bronchopulmonary exacerbations (BPE), microbiology and inflammatory markers (TNF-α, IL-8, IL-4, IFN-γ, neutrophil elastase activity and protein concentration) in the sputum and plasma.
85 children with CF (39-m) aged av. 12 years 2 months (6,0 -16,5 years) were assigned to three clinically comparable groups: I (n=30) - treated with clarithromycine (250 mg every other day for 12 months); II (n=25) - treated with azithromycin (250 mg three times a week for 18 months); III (control) (n=30) - on basic therapy.
Results: After 6 months of treatment, group I became statistically better than those in III group in the following tests: FEV1 (78.1±8.3% vs. 59.9±8.6%, p<0.01), FVC (84.5±7.2% vs. 68.1±7.8%, p<0.01) and Wt/Ht% (91.2±4.9% vs. 84.2±4.3%, p<0.05). Parameters in group I remained stable within the following 6 months. In group II, reliable differences with the control group were obtained only after 12 months: FEV1 (86.8±17.6% vs. 58.2±9.0%, p=0.05), FVC (89.0±18.8% vs. 66.7±7.8%, p=0.05), SBs (72.3±8.6 vs. 58.1±7.6,p=0.05) and remained stable to the end of the trial; after 18 months there was a difference in Wt/Ht% also (90.8±7.6% vs. 84.0±3.9%, p<0.05). There was a remarkable decrease in frequency of BPE in groups I and II.
We observed significant decreases in TNF-α (p=0.02), IL-8 (p=0.03), Il-4 (p<0.01), IFN-γ (p<0.001) in the sputum and a decrease of IL-4 in the plasma (p=0.002) in group I.
Conclusion: a beneficial effect of long-term low-dose therapy with macrolides is proved by functional and immunological changes.